# National Standard of the People's Republic of China

**GB 5009.5-2016**

# National Food Safety Standard

# Determination of Protein in Foods

## 食品安全国家标准

## 食品中蛋白质的测定


- Released on 2016-12-23
- Implemented on 2017-06-23
- Issued by NHFPC & CFDA

# Foreword

This standard replaces GB 5009.5-2010 -National food safety standard Determination of protein in foods, GB/T 14489.2-2008 Inspection of grain and oils-Determination of crude protein in oilseeds, GB/T 15673-2009 Determination of crude protein in edible mushroom, GB/T 5511-2008 Cereals and pulses-Determination of the nitrogen content and calculation of the crude protein content-Kjeldahl method, GB/T 9695.11-2008 Meat and meat products-Determination of nitrogen content, and GB/T 9823-2008 Inspection of grain and oils-Determination of total nitrogen content in plant oilseeds residues.

Compared with GB 5009.5-2010, the major changes of this standard are as follows:

— The conversion coefficient of protein in Annex A has been added.

# National Food Safety Standard

# Determination of Protein in Foods

## 1. Scope

This standard specifies the method for the determination of protein in foods.

Method I and Method II of this standard are applicable to the determination of protein in all kinds of foods; Method III applies to the determination of solid test samples with protein content higher than 10 g/100 g, including grains, beans, milk powder, rice powder and protein powder, *etc.*

This standard does not apply to the determination of the foods containing inorganic nitrogenous substances or non-protein organic nitrogenous substances.

### Method I Kjeldahl Nitrogen Determination

## 2. Principles

Under catalytic and heating conditions, the protein in food is decomposed to produce ammonia reacted with sulfuric acid to produce ammonium sulfate. Alkaline distilling makes ammonia free, absorb ammonia with boric acid and then titrate with sulfuric acid or hydrochloric acid standard volumetric solution. The nitrogen content is calculated based on the consumption of acid and then multiplied by the conversion coefficient to get the content of protein.

## 3. Reagents and Materials

3.1 Reagents

Unless otherwise specified, all the reagents in this method are analytically pure and the water is Grade 3 water as specified in GB/T 6682.

3.1.1 Copper sulfate ($CuSO_4 \cdot 5H_2O$).

3.1.2 Potassium sulfate (K2SO4).

3.1.3 Sulfuric acid (H2SO4).

3.1.4 Boric acid (H3BO3).

3.1.5 Methyl red indicator (C15H15N3O2).

3.1.6 Bromcresol green indicator (C21H14Br4O5S).

3.1.7 Methylene blue indicator (C16H18ClN3S·3H2O).

3.1.8 Sodium hydroxide (NaOH).

3.1.9 95% ethanol (C2H5OH).

3.2 Preparation of reagents

3.2.1 Boric acid solution (20 g/L): weigh 20 g of boric acid, dissolve and dilute to 1000 mL with water.

3.2.2 Sodium hydroxide solution (400 g/L): weigh 40 g of sodium hydroxide, dissolve with water, cool down, and then dilute to 100 mL.

3.2.3 [*c* (1/2 H2SO4)] 0.0500 mol/L sulfuric acid standard volumetric solution or [*c* (HCl)] 0.0500 mol/L hydrochloric acid titration volumetric solution.

3.2.4 Methyl red-ethanol solution (1 g/L): weigh 0.1 g of methyl red, dissolve and dilute to 100 mL with 95% ethanol.

3.2.5 Methylene blue-ethanol solution (1 g/L): weigh 0.1g of methylene blue, dissolve and dilute to 100 mL with 95% ethanol.

3.2.6 Bromocresol green-ethanol solution (1 g/L): weigh 0.1 g of bromocresol green, dissolve and dilute to 100 mL

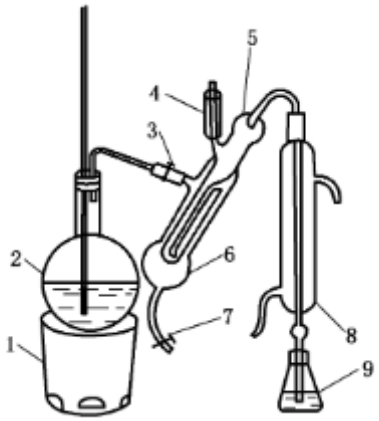
with 95% ethanol.

3.2.7 Mixed indicator A: mix 2 portions of methyl red-ethanol solution with 1 portion of methylene blue-ethanol solution, and this solution shall be ready before use.

3.2.8 Mixed indicator B: mix 1 portion of methyl red-ethanol solution with 5 portions of bromocresol green-ethanol solution, and this solution shall be prepared just before use.

## 4. Apparatus and Equipment

4.1 Balance: with sensitivity of 1 mg.
4.2 Nitrogen determination distillation apparatus: as shown in Fig.1.
4.3 Automatic Kjeldahl nitrogen analyzer.




Notes:
1——electric furnace;
2——steam generator (a 2-L flask);
3——screw clip;
4——small glass cup with rod-like glass stopper;
5——reaction chamber;
6——outer shell of reaction chamber;
7——rubber tube and screw clip;
8——condenser tube;
9——distillate receiving bottle.

**Fig. 1 Diagram of nitrogen determination distillation apparatus**

## 5. Analysis Procedures

5.1 Kjeldahl nitrogen determination

5.1.1 Test sample treatment: weigh a certain amount of absolutely mixed test sample (0.2 g~2 g for solid, 2 g~5 g for semi-solid and 10 g~25 g for liquid sample) approximately equal to 30 mg~40 mg of nitrogen), accurate to 0.001 g. Transfer into a 100mL, 250mL or 500mL dry nitrogen determination flask, add 0.4 g of copper sulfate, 6 g of potassium sulfate and 20 mL of sulfuric acid, shake gently, place a small funnel at the opening of the flask, and keep the flask at an inclined position (about $45^{o}$) on an asbestosed wire gauze with small meshes. Heat the flask carefully until all the contents are carbonized after the foam is completely stopped, then strengthen the heat power and keep the liquid in the flask at a slightly boiling state until the liquid becomes blue-green, clear and

transparent. Continue boiling the solution for 0.5~1 h. Take the flask away from the asbestosed wire gauze and cool down, and then add 20 mL water carefully. Cool down and transfer into a 100mL volumetric flask, rinse the flask with a small amount of water and transfer the washing solution into the same volumetric flask, then dilute to volume with water. Mix up for use. Meanwhile, perform the reagent blanktest.

5.1.2 Determination: install the nitrogen determination distillation apparatus according to Fig.1. Fill the steam generator with water to 2/3 of the volume, add several glass beads, a few drops of methyl red-ethanol solution and a few milliliters of sulfuric acid in order to ensure the water is acidic, heat to boil the water in the steam generator and keep the water boiling.

5.1.3 Add 10.0 mL of boric acid solution and 1~2 drops of mixed indicator A or B into the receiving bottle, and insert the bottom end of condenser pipe into the solution. According to the nitrogen content of test sample, accurately pipette 2.0~10.0 mL of the treating solution of test sample and pour into the reaction chamber through the small glass cup, wash the small glass cup with 10 mL of water and allow the washing solution flowing into the reaction chamber, and then the rod like glass plug is tightly packed. at once. Pour 10.0 mL of sodium hydroxide solution into the small glass cup, lift up the glass stopper to allow the solution flowing slowly into the chamber, stuff up the glass stopper at once, and make water seal. Clamp the screw clip and start distillation. Move the distillate receiving bottle 10 min later, allow the bottom of condenser tube leaving the liquid level, and keep distilling for 1 min. Rinse the outside of the bottom of the condenser tube with a small amount of water, and remove the distillate receiving bottle. Titrate the distillate with sulfuric acid or hydrochloric acid standard volumetric solution to the titration end point as soon as possible. The color of end point is greyish blue if mixed indicator A is used and the color of end point is light greyish red if mixed indicator B is used. At the same time, perform the reagent blank test.

5.2 Automatic kjeldahl nitrogen analyzer method

Weigh a certain amount of absolutely mixed sample (0.2 g~2 g for solid, 2 g~5 g for semi-solid and 10 g~25 g for liquid sample) (approximately equal to 30 mg~40 mg of nitrogen), accurate to 0.001 g, and place into a digestive tube. Add 0.4 g of copper sulfate, 6 g of potassium sulphate and 20 mL of sulfuric acid into the digestion furnace for digestion. When the temperature of digestion furnace reaches 420 ℃, continue digesting for 1 h, at this time the liquid in the digestive tube is in green and transparent state, take out and cool down, add 50 mL of water, and the automatic kjeldahl nitrogen analyzer (add sodium hydroxide solution, hydrochloric acid or sulfuric acid standard solution and boric acid solution containing mixed indicator A or B before use) can inject solution, distill, titrate and record titration data automatically.

## 6. Expression of Analysis Results

The content of protein in test sample is calculated according to Formula (1):

$$X = \frac{(V_1 - V_2) \times c \times 0.014\ 0}{m \times V_3/100} \times F \times 100 \quad \cdots\cdots(1)$$

Where,

$X$——the content of protein in the test sample, g/100 g;

$V_1$——the volume of the sulfuric acid or hydrochloric acid standard volumetric solution consumed by the test sample solution, mL;

$V_2$——the volume of the sulfuric acid or hydrochloric acid standard volumetric solution consumed by the reagent blank solution, mL;

$c$——the concentration of sulfuric acid or hydrochloric acid standard volumetric solution, mol/L;

0.0140——the mass of nitrogen equal to 1.0 mL of sulfuric acid [*c* ((1/2)$H_2SO_4$) = 1.000 mol/L] or hydrochloric acid [*c* (HCl) =1.000 mol/L] standard volumetric solution, g;
*m*——the mass of the test sample, g;
$V_3$——the volume of digestive solution pipetted, mL;
*F*——the coefficient of nitrogen conversion for protein, see Annex A for the nitrogen conversion coefficient of all kinds of foods;
100——conversion coefficient.
For the protein content equal to or higher than 1 g/100 g, the result shall keep three significant figures; while for the protein content lower than 1 g/100 g, the result shall keep two significant figures.

Notes: When only nitrogen content is detected, there is unnecessary to multiply the protein conversion coefficient F.

**7. Precision**

The absolute difference between the two independent determination results obtained under the repeated conditions shall be no more than 10% of the mean arithmetical value.

**Method II Spectrophotometry**

**8. Principles**

Under catalytic and heating conditions, the protein in food can be decomposed to produce ammonia, which is combined with sulfuric acid to produce ammonium sulfate. The ammonium sulfate can react with acetyl acetone and formaldehyde to yield a compound with yellow color, i.e. 3,5-diacetyl-2,6-dimethyl-1,4-dihydro-pyridine in sodium acetate-acetic acid buffer solution (pH 4.8). Measure the absorbance at the wavelength of 400 nm, and perform quantitative analysis compared with standards series. The result is multiplied by the conversion factor, which is the protein content.

**9. Reagents and Materials**

9.1 Reagents
Unless otherwise specified, all the reagents used in the method are analytically pure, and the water is Grade 3 water specified in GB/T 6682.
9.1.1 Copper sulfate ($CuSO_4 \cdot 5H_2O$).
9.1.2 Potassium sulfate ($K_2SO_4$).
9.1.3 Sulfuric acid ($H_2SO_4$): guaranteed reagent.
9.1.4 Sodium hydroxide (NaOH).
9.1.5 P-nitrophenol ($C_6H_5NO_3$).
9.1.6 Sodium acetate ($CH_3COONa \cdot 3H_2O$).
9.1.7 Anhydrous sodium acetate ($CH_3COONa$).
9.1.8 Acetic acid ($CH_3COOH$): guaranteed reagent.
9.1.9 37% formaldehyde (HCHO).
9.1.10 Acetyl acetone ($C_5H_8O_2$).

9.2 Preparation of reagents

9.2.1 Sodium hydroxide solution (300 g/L): weigh 30 g of sodium hydroxide, dissolve with water, cool down and dilute to 100 mL.

9.2.2 P-nitrophenol indicator (1 g/L): weigh 0.1 g of p-nitrophenol, dissolve in 20 mL of 95% ethanol, and dilute to 100 mL with water.

9.2.3 Acetic acid solution (1 mol/L): measure 5.8 mL of acetic acid, and dilute to 100 mL with water.

9.2.4 Sodium acetate solution (1 mol/L): weigh 41 g of anhydrous sodium acetate or 68 g of sodium acetate, dissolve and dilute to 500 mL with water.

9.2.5 Sodium acetate-acetic acid buffer solution: measure 60 mL of sodium acetate solution and 40 mL of acetate acid solution and mix absolutely. The pH of the buffer solution is 4.8.

9.2.6 Color developing agent: mix 15 mL of formaldehyde with 7.8 mL of acetyl acetone, dilute to 100 mL with water, and shake violently to mix absolutely (stand and stabilize it at room temperature for 3 d).

9.2.7 Ammonia nitrogen standard stock solution (as nitrogen) (1.0 g/L): weigh 0.4720 g of ammonium sulfate (pre-dried at 105℃ for 2 h), dissolve with water and transfer into a 100mL volumetric flask, dilute to volume, and mix absolutely. Each 1 mL of the obtained solution is equal to 1.0 mg of nitrogen.

9.2.8 Ammonia nitrogen standard working solution (0.1 g/L): transfer 10.00 mL of ammonia nitrogen standard stock solution into a 100mL volumetric flask, dilute to volume with water, and mix absolutely. Each 1 mL of the obtained solution is equal to 0.1 mg of nitrogen.

## 10. Apparatus and Equipment

10.1 Spectrophotometer.

10.2 Electro-heated thermostatic water bath: with temperature controlled within 100℃± 0.5℃.

10.3 Glass colorimetric tubes with stopper, with nominal capacity of 10mL.

10.4 Balance: with sensitivity of 1 mg.

## 11. Analysis Procedures

11.1 Digestion of test sample

Weigh a certain amount of absolutely mixed test sample [0.1 g~0.5 g (accurate to 0.001 g) for solid sample, 0.2 g~1 g (accurate to 0.001 g) for semi-solid sample and 1 g~5 g (accurate to 0.001g) for liquid sample],move into a 100mL or 250mL dry nitrogen determination flask, add 0.1 g of copper sulfate, 1 g of potassium sulfate and 5 mL of sulfuric acid, shake absolutely, place a small funnel at the opening of the flask, and keep the flask at an inclined position (about 45$^{o}$) on an asbestosed wire gauze with meshes. Heat the flask slowly until all the contents are carbonized and the bubbles stop, then strengthen the heat power and keep the liquid in the flask at a slightly boiling state until the liquid becomes blue-green and clear and transparent. Continue heating for 0.5 h. Take the flask away from the asbestoses wire gauze and cool down, and then add 20 mL of water carefully. Cool down and transfer into a 50mL or 100mL volumetric flask, rinse the nitrogen determination flask with a small amount of water and merge the washing solution into the same volumetric flask, then dilute to volume with water. Mix absolutely for use. At the same time, follow the same method for reagent blank test.

11.2 Preparation of test solution

Pipette 2.00 mL~5.00 mL of the digestive solution of test sample or reagent blank solution into a 50mL or 100mL volumetric flask, add 1~2 drops p-nitrophenol indicator, and shake absolutely. Dropwise add sodium hydroxide solution to neutralize the solution until it turns to yellow. Then dropwise add acetate acid solution until the solution turns to colorless. Dilute to volume with water and mix absolutely.

11.3 Plotting of standard curve

Pipette 0.00, 0.05, 0.10, 0.20, 0.40, 0.60, 0.80 and 1.00 mL of ammonia nitrogen standard working solution (equal to 0.00, 5.00, 10.0, 20.0, 40.0, 60.0, 80.0 and 100.0 μg of nitrogen) into 10mL glass colorimetric tubes, respectively. Add 4.0 mL of sodium acetate-acetic acid buffer solution and 4.0 mL of color developing agent, dilute to volume with water, and mix absolutely. Place the tubes in a water bath at 100 ℃ and heat for 15 min. Take out water and cool down to room temperature. Transfer into 1mL cuvettes. Take the tube without ammonia nitrogen standard working solution as reference, measure the absorbance at the wavelength of 400 nm. Draw standard curve or calculate the linear regression equation based on the absorbance of each standardsolution.

11.4 Determination of test sample

Pipette 0.50 mL~2.00 mL of test sample (approximately equal to nitrogen content of less than 100 μg) and the same volume of reagent blank solution into 10mL colorimetric tubes, respectively. Add 4.0 mL of sodium acetate-acetic acid buffer solution and 4.0 mL of color developing agent, dilute to volume with water, and mix absolutely. Place the tubes in a water bath at 100 ℃ and heat for 15 min. Take out water and cool down to room temperature. Transfer into 1mL cuvettes. The absorbance at a wavelength of 400 nm is measured with a tube containing no ammonia nitrogen standard working solution as a reference.. Perform quantitative analysis by comparing the absorbance of test sample with standard curve or calculate the protein content by plugging the absorbance of test sample into the linear regression equation.

## 12. Expression of Analysis Results

The content of protein in the test sample is calculated according to Formula (2):

$$X = \frac{(C - C_0) \times V_1 \times V_3}{m \times V_2 \times V_4 \times 1\,000 \times 1\,000} \times 100 \times F \quad \cdots\cdots (2)$$

Where,

$X$——the content of protein in the test sample, g/100 g;

$C$——the content of nitrogen in the test solution applied for determination, μg;

$C_0$——the content of nitrogen in the reagent blank solution applied for determination, μg;

$V_1$——the volume of the digestive solution of test sample after being diluted to volume, mL;

$V_3$——the total volume of test sample solution, mL;

$m$——the mass of the test sample, g;

$V_2$——the volume of digestive solution applied for the preparation of test sample solution, mL;

$V_4$——the volume of test sample solution applied for determination, mL;

1000——conversion coefficient;

100——conversion coefficient;

$F$——the coefficient of nitrogen conversion for protein.

For the protein content equal to or higher than 1 g/100 g, the result shall keep three significant figures; while for the protein content lower than 1 g/100 g, the result shall maintain two important figures.

## 13. Precision

The absolute difference between the two independent determination results obtained under the repeatability conditions shall not exceed 10% of their mean arithmetical value.

## Method III Combustion method

### 14. Principles

Test sample is combusted at 900 ℃~1200 ℃, producing gas mixture, among which the carbon, sulfur and other interfering gases and salts are absorbed by absorption tube, and nitrogen oxides are absolutely reduced to nitrogen gas, which is decide by thermal conductivity detector (TCD).

### 15. Apparatus and Equipment

15.1 Nitrogen/protein analyzer;
15.2 Balance: with sensitivity of 0.1 mg.

### 16. Analysis Procedures

Weigh 0.1 g~1.0 g of absolutely mixed test sample (accurate to 0.0001 g) according tothe instruction manual of the analyzer. Wrap the sample with tin foil and place onto the sample plate. Inject the sample into the combustion furnace (900 ℃~1200 ℃), and the sample will be combusted fully in high pure oxygen (with purity ≥99.99%). The reaction products in the combustion furnace ($NO_x$) are fed to the reduction furnace (800 ℃) by carrier gas carbon dioxide or helium and after the products are reduced to nitrogen gas in the reduction furnace, determine the content of nitrogen gas.

### 17. Expression of analysis results

The content of protein in the test sample is calculated according to Formula (3):

$$X = C \times F \qquad \cdots\cdots (3)$$

Where,
*X*——the content of protein in the test sample, g/100 g;
*C*——the content of nitrogen in the test sample, g/100 g;
*F*——the coefficient of nitrogen conversion for protein.
The result shall keep three significant figures.

### 18. Precision

The absolute difference between the two independent determination results obtained under the repeatability conditions shall not exceed 10% of their mean arithmetical value.

### 19. Other information

The limit of detection of Method I in this standard is 8 mg/100 g when the sampling amount is 5.0 g.
The limit of detection of Method II in this standard is 0.1 mg/100 g when the sampling amount is 5.0 g.

## Annex A

## Nitrogen-to-protein conversion coefficients in common foods

The nitrogen-to-protein conversion coefficients in common foods are shown in Table A.1.

**Table A.1 Conversion coefficients of protein**

| Food categories | | Conversion coefficients | Food categories | | Conversion coefficients |
|---|---|---|---|---|---|
| Wheat | Whole wheat flour | 5.83 | Rice and rice noodles | | 5.95 |
| | Wheat bran | 6.31 | Eggs | Eggs (whole) | 6.25 |
| | Wheat germ | 5.80 | | Yolk | 6.12 |
| | Wheat germ flour, rye, common wheat, flour | 5.70 | | Egg white | 6.32 |
| Oat, barley, rye flour | | 5.83 | Meat and meat products | | 6.25 |
| Millet, naked barley | | 5.83 | Animal gelatin | | 5.55 |
| Corn, black wheat, feed wheat, sorghum | | 6.25 | Pure milk and pure milk products | | 6.38 |
| Oil plants | Sesame seeds, cottonseeds, sunflower seeds, castors, safflower seeds | 5.30 | Compound formula products | | 6.25 |
| | Other oil plants | 6.25 | Casein | | 6.40 |
| | Rapeseeds | 5.53 | | | |
| Nut, seeds | Brazilnuts | 5.46 | Collagen | | 5.79 |
| | Peanuts | 5.46 | Beans | Soybean and its rough processing products | 5.71 |
| | Almonds | 5.18 | | Soybean protein products | 6.25 |
| | Walnuts, hazelnuts, coconuts, *etc*. | 5.30 | Other foods | | 6.25 |